1. The Beginnings. A Multicentric Disease

"We cannot identify the unknown aspects of modern life that lead to atherosclerosis until we know the true nature of the characteristic atherosclerotic lesion."
Earl Benditt, [12]

The Injurious Agent

Throughout this comprehensive study the term "Injurious Agent" (IA) will be used to refer to any agent or any process, singly or in combination, that might cause cellular dysfunction or injury to the artery wall, resulting in atherosclerosis.

Response-to-Injury

The response-to-injury hypothesis states that the initial event in the pathogenesis of atherosclerosis is injury to the endothelium [1]. A variety of IAs produce an inflammatory response in which leucocytes, primarily monocytes, migrate to the area of injury [2]. The result is retention and oxidation of lipoproteins and transformation of monocytes into macrophages that ingest lipid, particularly oxidized low density lipoproteins (LDL). These form the fatty streak that is an early objective sign of atherosclerosis [2]. Important considerations in this theory are the precise nature of the IA, and the sequence of events that lead to the retention of lipid. Several studies in experimental animals have demonstrated that lipid retention occurs before the monocytes migrate into the intima [3], showing that the monocyte is not the cause of the lipid retention [4,5]. What, then, is the cause or the mechanism of the lipid retention?

Proteoglycans and the Extracellular Matrix

Previous studies demonstrated that the initial lesion in atherosclerosis is asymmetrical intimal thickening, the result of increased production of sulfate-containing proteoglycans (PGs) - primarily Chondroitin Sulfate Proteoglycans (CSPG) and other forms of extracellular matrix (ECM) - by resident intimal smooth muscle cells (SMCs) in a focal area of the arterial wall [6–9]. The IA, directly or indirectly, enters the arterial wall from the circulating blood, and then either stimulates or enters the resident SMC, the principal source of vascular PGs [10], to produce increased amounts of PGs and ECM. Walton [8] showed this mucoid thickening of the intima occurs before lipid infiltration and is composed primarily of collagen, PGs, and ECM. Thus, although lipid accumulation in the artery wall is considered an early event in atherosclerosis, lipid retention is not the initiating event, and the fatty streak is not the first sign of atherosclerotic injury [11,12]. This initial intimal thickening is not characterized by hypercellularity or proliferation of SMCs [13], as is seen in other types of vascular injury [14], but rather by relative acellularity, apparently due to the increased amounts of PGs and ECM without associated SMC proliferation [15]. The relative acellularity noted in these early lesions is not believed to be due to massive cell death of resident intimal cells [16]. Increased production of PG and ECM, without an increase in the number of SMCs, is an unusual response to injury, suggesting a specific type of IA and/or a specific type of injury or effect on the SMC [17,18].

Whether the increase in PGs and ECM is a pathologic response and, therefore, to be prevented, or is a physiologic defensive, protective, or reparative

response to the IA is not known [6,10]. The fact that these intimal thickenings develop very early after wall injury and before lipid accumulation suggest this is a protective, healing, or defensive response [1,6]. This view is supported by the knowledge that CSPG is required and is the predominant PG in normal wound repair [9]. However, if this is a physiologic defense, it fails badly because the IA agent is not halted, proliferation of PGs and ECM continues, and resolution, healing, and stabilization do not occur. The disease continues to progress. In addition, if the production of PGs and ECM is a physiologic defense, why is lipid retained?

The ECM is a visco-elastic material containing primarily CSPG, a biochemically active scaffold that regulates arterial permeability, filtration, transport of plasma constituents, and regulation of wall metabolism and function [10]. The increased amount of PGs produced by the SMC in response to various growth factors associated with atherosclerotic injury have much longer side chains and form larger aggregates than do the PGs normally found in the artery wall [10,13,19]. Thus, there is not only an increase in the PGs and ECM produced, but a change in the structure of the PGs in the areas of atherosclerotic injury. This change in PG structure is believed to alter the metabolic properties and biochemical function of the PGs and ECM, resulting in a disturbance in the transfer of substrates through the zone of injury, particularly alteration of interactions with lipoproteins [10,13,19,20]. These structural and functional changes in the PGs as well as their turnover rate, are directly related to the rate of retention of lipid in the interstices of the ECM [8,9]. The alteration in structure and the increased production of PGs suggest a pathologic component of the disease process, produced and altered, not as a physiologic defense [2,8], but for the specific purpose of retaining lipid, particularly LDL.

Adaptive Intimal Thickening

Stary, et al. [21], believe many asymmetric intimal thickenings, termed Adaptive Intimal Thickening, reflect a physiologic adaptive response to hemodynamic stress. They found this thickening at points of arterial bifurcation in infant human beings and animals. The authors point out that such physiologic thickenings may also be the site of atherosclerotic plaques. It may be difficult to distinguish thickenings that are physiologic adaptations from those that are pathologic, particularly in the early stages of atherosclerosis. These adaptive intimal thickenings are rich is PGs [21]. Evidence of lipid retention, then, is a primary feature that distinguishes physiologic thickening from pathologic atherosclerosis [21]. The presence of intimal thickening at points of bifurcation supports the view that these lesions are an adaptation to hemodynamic stresses, but the occurrence of the same lesions in areas without bifurcations, Figure 1, or areas of low or relatively low hemodynamic stress, suggests other factors are also involved. These other additional factors may be acting independently or in conjunction with hemodynamic stresses to transform adaptive intimal thickening into atherosclerotic lesions.

Whether intimal thickenings are initially physiologic or pathologic, they reflect tissue proliferation to some sort of IA at any age. The fact that some adaptive intimal thickenings progress on to atherosclerotic lesions indicates that all such thickenings may possess the potential to do so and to become a vulnerable site for the IA to enter the wall [2,11].

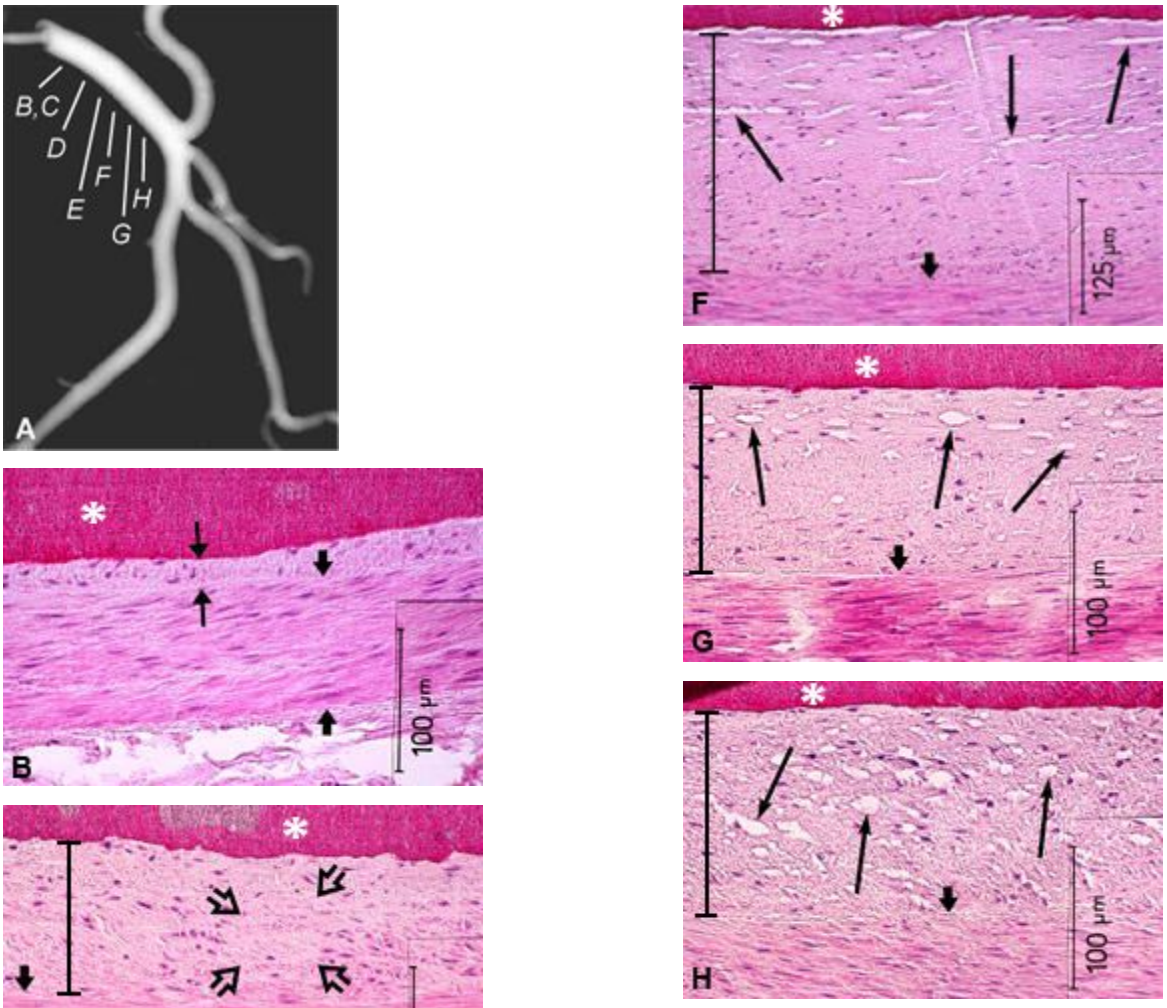


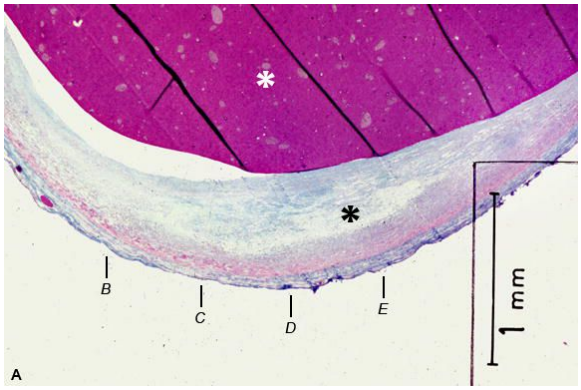
Figure 1: **A**, Dissected left coronary artery from a 31-year-old male who died of head injuries. The artery shows no radiographic evidence of atherosclerosis. **B**-**H** are contiguous segments of the main left coronary artery, as labeled in **A. B**, Normal appearing intima (thin arrows) and media. **C**, Same coronary segment as **B**, but directly opposite on the other side of the lumen. The intima here is thicker (bracket), with a small focus of relative acellularity (open arrows). **D**, Slightly increased intimal thickening (bracket) and an increased area of acellularity (open arrows) compared to **C. E**, Marked increase in intimal thickness (bracket) with loss of tissue and cells (open arrows) consistent with focal degeneration. Lipid-laden SMCs (long arrows) surround this area of degeneration. **F**, Further increase in intimal thickness (bracket) with areas of acellularity and lipid-laden SMCs (long arrows). No areas of degeneration can be identified. **G** & **H**, Intimal thickening (bracket) is decreasing distally from **F**, but with more prominent and more numerous lipid-laden SMCs (long arrows). White asterisk = lumen, fat arrows = media. Hematoxylin & Eosin (H & E) stain in all photos.

Early Atherosclerotic Lesions

Figure 1 shows early changes of atherosclerosis in a 31-year-old white male who died of non-cardiac causes. Asymmetric intimal thickening begins near the left coronary ostia (Figures 1B, 1C), and continues through all contiguous coronary segments, ending at Figure 1H. Focal areas of relative acellularity can be identified in Figures 1C–1H, consistent with increased production of PGs and ECM by resident intimal cells, presumably in response to wall injury [21]. Lipid retention in the form of lipid-laden macrophages or extracellular lipid, or evidence of tissue injury are not evident in unaffected intima (Figure 1B) nor in mild intimal thickening (Figure 1C). This finding supports the view that intracellular or extra-cellular lipid deposition does not occur in the normal artery wall, but only follows the development of intimal thickening [2,8]. The asymmetric intimal thickening involving only a portion of the luminal circumference indicates that the injury is focal and that the IA is present and presumably active at this particular site. Why the IA enters or affects the wall at a particular site has not been fully worked out, but it is probably related to local susceptibility, increased vulnerability, and/or focal injury to the endothelium by a various agents [1,2].

The presence of asymmetric thickening to a similar degree through contiguous coronary segments, as shown in Figures 1C–1H, suggests these thickenings are part of one continuous area of injury extending in a longitudinal direction [21,22]. If this assessment is correct, do these contiguous thickenings reflect injury from a single IA that has spread from a single focal site in a proximal and distal direction, or do they represent multiple, separate foci of injury to the same or different IAs? If we postulate that one agent caused all these lesions from one focus, is it possible to identify the initial injury site histologically? In theory, the initial site of injury should show the most advanced histologic changes because the IA has been present and active for a longer period of time than in adjacent sections. Figure 1E shows the most advanced changes in terms of tissue degeneration, cell loss, and lipid accumulation, located approximately midway between the first proximal intimal thickening (Figure 1C), and the distal thickening in Figure 1H. Therefore, Figure 1E could be the site of initial injury and the development of intimal thickening, proximally and distally, may reflect direct spread of the IA agent in both directions. A review of Figures 1C–1H confirms that the amount of intimal hickening and the severity of the degenerative changes tend to decrease in both directions from Figure 1E.

The histologic changes observed in Figures 1C and 1H may represent the leading edge of the injury that is spreading longitudinally from the central site in Figure 1E. Furthermore, if the IA can spread longitudinally then it may also spread circumferentially, with the leading edge of the injury expanding in the direction of the plaque shoulder (Figure 2). If this is the correct interpretation of these histologic changes, it means an IA can establish a foothold in the artery wall in spite of defensive responses. It then has the potential to spread by direct contiguity in all directions from this central focus.



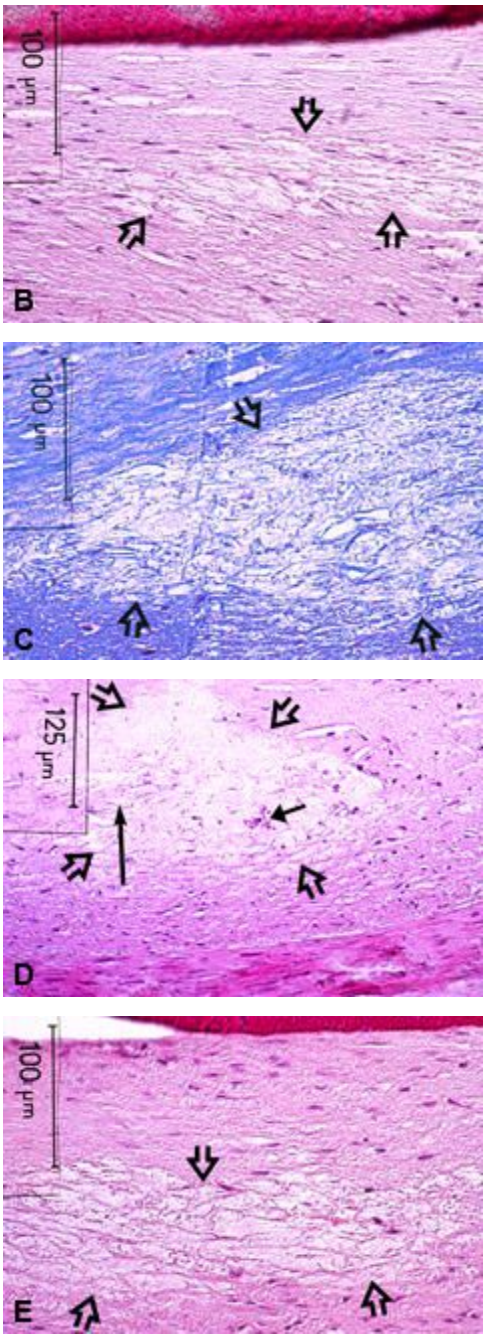


Figure 2: Coronary section taken from the same left coronary artery illustrated in Figure 1, approximately 1 centimeter distal to Figure 1H. **A**, Low-power view of a small asymmetric plaque with a central core (black asterisk). Martius Scarlet Blue (MSB) stain. White asterisk = lumen. **B** - **E**, High-power views of different parts of the plaque corresponding to the letters shown in **A. B**, Focal area of tissue degeneration, cell loss, and lipid retention (open arrows) near one shoulder of the plaque. H & E stain. **C**, Midway between the shoulder and the center of the plaque, showing the size of the degenerative area (open arrows) is larger than in **B**. MSB stain. **D**, Central area of the plaque showing focal degeneration (open arrows) and frank necrosis, including the presence of cholesterol crystals (long arrow) and calcification (short arrow). H & E stain. **E**, Area near the other plaque shoulder with similar amount of tissue degeneration (open arrows) as shown in **B**. H & E stain.

Advanced Degeneration

Figure 2, illustrates, in the same artery of the same patient, another, more advanced focus of intimal thickening and plaque formation, but distal to that shown in Figure 1A. The plaque in Figure 2A is the largest, most advanced atherosclerotic plaque in this artery, but it is still insignificant in terms of luminal stenosis. The central area of this plaque (Figure 2D), shows advanced degeneration with focal necrosis of tissue, loss of both ECM and cells, lipid-laden SMCs, lipid infiltration, formation of cholesterol crystals, and a tiny focus of calcification. The necrosis and calcification indicate this is an “advanced” lesion, according to the classification of the American Heart Association’s Committee on Vascular Lesions [15]. According to this classification, any lesion, regardless of luminal stenosis, that contains an atheroma and/or a fibrolipid plaque and calcification is considered to be an advanced lesion.

The advanced degenerative changes in Figure 2D extend toward each plaque shoulder, where the plaque meets the normal arterial wall, the area of degeneration and necrosis becoming progressively smaller and less severe toward the plaque shoulder, Figures 2B, 2E. Again, as in Figure 1, the IA appears to be spreading by direct contiguity, only this time in a circumferential direction toward the plaque shoulder. The tissue immediately surrounding the central core (Figure 2E) appears devoid of cells, suggesting that these cells were either destroyed by substances contained within the necrotic core, have undergone apoptosis or died of some other consequence of the disease process [23,24]. Progressive cell loss is characteristic of

advanced lesions and, with cell death, degeneration, necrosis, and formation of a necrotic core follow [23–26].

The plaque in Figure 2A extends proximally into the adjacent coronary section and distally into two additional segments, again suggesting active longitudinal spread of the IA. The remainder of the coronary artery distal to the segment illustrated in Figure 2 showed two additional focal, widely separated asymmetric intimal thickenings, similar to, but less severe than those shown in Figure 1. Altogether there were four separate lesions separated by normal arterial wall in this one artery. Wilens [11] noted that IAs can attack the wall at multiple sites, setting in motion atherosclerotic disease at multiple points, emphasizing atherosclerosis is a multicentric disease.

In Review

The IA causing atherosclerosis appears to enter at a focal point in the artery wall. In some way it stimulates the resident intimal SMC to produce increased amounts of an abnormal form of PGs, resulting in asymmetric intimal thickening and lipid retention. Asymmetric intimal thickenings are ubiquitous throughout the coronary tree because atherosclerosis is multicentric in origin and the IA, present in circulating blood, may enter the wall at any vulnerable point. The production of an abnormal form of PGs appears to be a pathologic component of the disease process, produced specifically to retain lipid. The IA appears to establish a locus or focus of injury and then spreads in all directions from this central focus, to contiguous areas within the intimal layer. Lipid-laden SMC are an early, but not the earliest sign of atherosclerosis. Degeneration, necrosis and calcification of plaque tissue can occur very early in plaque development. The defensive responses, whatever they may be, appear to be unable to halt, sequester, or neutralize the IA or to effect healing and resolution of the injured area.

Unanswered Questions

What is the IA and what is the mechanism of injury? What kind of an IA can enter the wall and injure or stimulate resident SMC to produce excess amounts of abnormal PGs without stimulating the proliferation of SMC or attracting other SMC from the media? Does the IA enter the SMC without injuring the extracellular matrix? Why does this particular form of PGs retain lipid, when the normally present PGs do not? What is the relationship between the IA and the retained lipid? What is the mechanism by which the IA spreads throughout the intima? Why do some mucoid swellings degenerate and become necrotic very early in plaque development while others do not? These questions will be explored in subsequent chapters.